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Original Article

ANTIBACTERIAL ACTIVITY OF THE METABOLITES OF *ASPERGILLUS CHEVALIERI* AND *TRICHODERMA HARZIANUM*

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ABSTRACT

Objective: This study sought to preliminarily investigate the inhibitory effect of metabolites of *Aspergillus chevalieri* and *Trichoderma harzianum* on a number of pathogenic bacteria.

Methods: The agar well diffusion method was employed to determine the antimicrobial activity of the fungal metabolites. The test microorganisms were *Enterococcus faecalis,* methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa.*

Results: Both metabolites had broad-spectrum antibacterial activity. All the test organisms were susceptible to the *A. chevalieri* metabolites except for *S. typhi.* Both *S. typhi* and *E. faecalis* were however not susceptible to *T. harzianum* metabolites. *P. aeruginosa* was highly susceptible to both metabolites with the highest zone of inhibition of 26 mm for the stock metabolite. This activity was comparable to the standard, 10 µg/ml of ciprofloxacin.

Conclusion: Metabolites of *A. chevalieri* and *T. harzianum* exhibited broad-spectrum activity, and this can be exploited as a source for novel antibiotics.

Keywords: Antibacterial activity, Antibiosis, *Aspergillus chevalieri, Trichoderma harzianum,* Culture metabolites

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INTRODUCTION

Infectious diseases caused by bacteria, fungi, and protozoa are common and widespread. They are the primary causes of morbidity, mortality, and a social and economic hindrance for millions of people [1]. According to the World Health Organization (WHO), over 9.5 million people die yearly due to infectious diseases [2]. The rapid emergence of resistant microbial strains has even more so greatly endangered the efficacy of existing antibiotics [3], coupled with the slow rate drug development, the world is unable to keep up with the fight against resistant microbial strains [4]. Worldwide, the emergence of antimicrobial-resistant bacterial strains is increasingly limiting the potency of current drugs, and significantly causing treatment failures [5, 6]. This has necessitated the fervent need to search for new antibiotics from natural products [7, 8]. Fungi were among the first sources of antibiotics [9] known to man. The discovery and development of the penicillins and cephalosporins from fungal sources, till date, remains some of the most important antibiotics [10] to humankind.

The Fungi Kingdom is the second largest [11] in species, and its members frequently employ the technique of antibiosis in combating bacteria. Antibiosis is the inhibition of one microorganism by the metabolic product of another. The metabolite penetrates the cell and inhibits it by chemical toxicity [12]. Fungi are a rich source of several bioactive compounds, including antibiotics [11]. In that respect, the fungi, *Trichoderma harzianum* and *Aspergillus chevalieri*, which are widely available and whose related species are widely known to have the ability to exhibit antibiosis were chosen.

A. chevalieri, belongs to the family Trichocomaceae. It is a storage mould affecting the germination of grains [13]. Invasion of such stored grains is under both hermetic and non-hermetic conditions [14]. *A. chevalieri* is known for its ability to grow at extremely low water activities [15]. In humans, *A. chevalieri* is an opportunistic pathogen which causes skin infections [16]. *Aspergillus* species on the other hand, are ubiquitous molds [17], being the most prevalent fungi present in all soils. Many species in this genus are characterized as opportunistic avirulent plant symbionts [18]. Several strains of *Trichoderma* such as *T. harzianum, T. viride* and *T. hamatum* have been developed as biocontrol agents against fungal diseases of plants [19]. *Trichoderma* species are also known to exhibit antimicrobial activity against a significant number of bacteria, yeasts, and filamentous fungi [20]. These species release antibiotics and other chemicals that are harmful to pathogens. The aim of this study was to therefore evaluate the inhibitory effect of metabolites of *A. chevalieri* and *T. harzianum* on a number of pathogenic bacteria.

MATERIALS AND METHODS

Chemicals and reagents

Potato Dextrose Broth (PDB), Nutrient agar and Nutrient Broth with reference ciprofloxacin, were all obtained from (Oxoid Limited, UK). All other reagents were obtained from standard suppliers.

Culture metabolite preparation

To obtain the culture metabolites of *Aspergillus chevalieri,* and *Trichoderma harzianum*, 3 cm agar disc of these fungi was inoculated into separate 500 ml Erlenmeyer flask containing 250 ml of sterile Potato Dextrose Broth (PDB). They were prepared in triplicates for each of the test fungi. These were incubated at room temperature for 7 d. The medium was then filtered by suction and pooled together. The collected filtrate was further filtered through a sterile Acrodisc® Millipore filter 0.2 µm (Gelman Sciences, USA). This served as the stock.

Test organisms

The bacteria and fungi used for the experiments were obtained from the Department of Pharmaceutics and Microbiology of the School of Pharmacy (UGSOP), University of Ghana. The gram-positive bacteria employed in this study were *Enterococcus faec*alis and methicillin-resistant *Staphylococcus aureus* (MRSA). The gram-negative bacteria employed were *Salmonella typhi, Escherichia coli,* and *Pseudomonas aeruginosa*.

Preparation of test solutions

The undiluted filtrate served as the stock, from which different dilutions were made. This was done by performing serial dilutions of

2 ml of the stock solution with 2 ml of sterile Millipore water in sterile 6 well plates. The final test solutions were the stock, 1 in 2 dilution, 1 in 4 dilution, and 1 in 8 dilution.

Antibacterial activity determination

The antibacterial activity was evaluated using the agar well diffusion method [21]. Twenty millilitres of sterile molten nutrient agar, stabilized at 45 °C for 15 min were seeded with 50µl of a 24 hour culture of test bacteria, and aseptically poured into a sterile petri dish and allowed to set. The bacteria and fungi cultures were standardized to a 0.5 McFarland tube. Four wells (8 mm in diameter) equidistant from each other were created with a sterile cork borer (number 5). The wells were then filled with 100µl of the stock, 1 in 2, 1 in 4, and 1 in 8 dilutions of the fungal metabolites. The plates were pre-incubated for 30 min at room temperature. This was to allow diffusion of the metabolites. The plates were then further incubated at 37 °C for 24 h [22, 23]. Reference antibiotic, ciprofloxacin (10 µg/ml) was used as the control. The procedure was performed in it triplicates and the mean zones of growth inhibition were determined.

RESULTS

The metabolites from *A. chevalieri* inhibited the growth of *S. typhi*, and *P. aeruginosa* up to the 1 in 4 dilution. The higher concentration of 1 in 2 dilution of this same metabolite demonstrated activity against *MRSA* and *E. facecalis*. No activity was seen against *S. typhi* in the concentrated extract. The 1 in 8 dilution of *T. harzianum* metabolite was active against *E. coli* and *P. aeruginosa*, while activity was seen against *MRSA* up to the 1 in 4 dilution, however this metabolite was neither active against *S. typhi* nor *E. faecalis*. The culture metabolites of *A. chevalieri* and *T. harzianum* had broad-spectrum antimicrobial activity against both Gram positive and Gram negative bacteria. That of *T. harzianum* metabolites was more active at the highest dilution of 1 in 8 than *A. chevalieri* metabolites. Detailed results are displayed in tables 1 and 2. Fig. 1 is the growth of the two fungi under investigation, on potato dextrose agar.

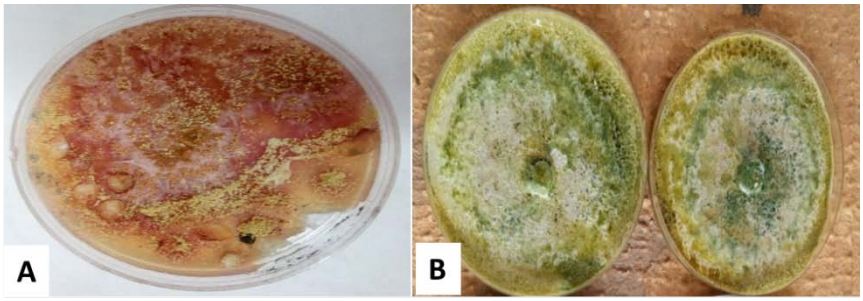

Fig. 1: *Aspergillus chevalieri* and *Trichoderma harzianum* on potato dextrose agar

Table 1: Antimicrobial activity of *A. chevalieri*

Test organism	Dilutions	Zone of inhibition (mm)	Ciprofloxacin (10 µg/ml)
E. coli	Stock	19.33±2.31	38±1.9
	1in 2	17.00±1.00	
	1in 4	13.00±0.00	
Ps. aeruginosa	Stock	25.50±2.12	28±3.8
	1 in 2	21.50±0.71	
	1 in 4	16.00±0.00	
S. typhi	Stock	-	42±2.9
	1 in 2	-	
MRSA	Stock	18.67±1.53	37±2.0
	1 in 2	13.33±1.15	
E. faecalis	Stock	19.00±0.00	24±3.2
	1 in 2	17.00±0.00	

n=3, data given is mean±SEM

Table 1: Antimicrobial activity of *T. harzianum*

Test organism	Dilutions	Zone of inhibition (mm)	Ciprofloxacin (10 µg/ml)
E. coli	Stock	27.00±2.65	38±1.7
	1 in 2	22.33±4.04	
	1 in 4	20.00±0.00	
	1 in 8	15.00±0.00	
Ps. aeruginosa	Stock	27.50±2.12	28±3.0
	1 in 2	25.00±2.83	
	1 in 4	21.50±0.71	
	1 in 8	16.00±0.00	
S. typhi	Stock	-	42±2.7
	1 in 2	-	
MRSA	Stock	24.33	37±2.5
	1 in 2	19.33	
	1 in 4	15.00	
E. faecalis	Stock	-	24±3.1
	1 in 2	-	

n=3, data given is mean±SEM

DISCUSSION

Increasing levels of multidrug resistant microorganisms, coupled with the slow rate of drug development has led to the dire need for new antibiotics [24]. Natural products are known to be rich sources of antimicrobial drugs. They account for over two-thirds of clinically important antibiotics and half of anticancer medicines in use [25]. The inhibition of the pathogenic organism's *P. aeruginosa, MRSA, E. coli* and *E. facecalis* is indicative of the presence of antimicrobially active compounds in these metabolites. The synthesis of antimicrobial compounds by fungal strains is a well-known phenomenon. Fungi strains are known to produce secondary metabolites that aid their virulence and survival against other microorganism [26]. *A. chevalieri* metabolites inhibited both Gram positive and Gram negative bacteria, hence it can be described as having broad spectrum antibacterial activity. These results agrees with previous reports that *Aspergillus* species are widely known to produce antibiotics. The antibiotics produced are components of the metabolites and are capable of inhibiting the growth of other microorganisms [11]. Aspergillic acid, penicillic acid and fumagillin [17] are well-known examples of such antibiotics. The metabolites showed significant activity against *P. aeruginosa* which was comparable to ciprofloxacin, the reference antibiotic. *P. aeruginosa* is one of the multidrug-resistant bacteria which is hard to treat. *P. aeruginosa* is known to have resistance to intermediate susceptibility to at least one antibiotic. This high level of resistance is attributable to the multiple intrinsic resistance mechanisms that *P. aeruginosa* may express, which include beta-lactamase production, efflux-mediated and porin-related resistance, and target site modification [24]. These mechanisms are often present in combination, causing a broad range of antibiotics to be rendered ineffective against a given *P. aeruginosa* isolate [27]. In that respect, these fungal metabolites can therefore be exploited to develop new antibiotics. *T. harzianum* metabolites also inhibited the growth of *E. coli. P. aeruginosa, MRSA* but not *E. faecalis* and *S. typhi*. These results are in accordance with other published data [18, 19, 28]. The suggested mechanisms for biocontrol of *Trichoderma* species of plant pathogens include antibiosis, lysis, competition, and mycoparasitism [29]. *Trichoderma* species are also known to exhibit antimicrobial activity against a significant number of bacteria, yeasts, and filamentous fungi [20]. These species release antibiotics and other chemicals that are harmful to pathogens through antibiosis.

CONCLUSION

In conclusion, *Aspergillus chevalieri* and *Trichoderma harzianum* metabolites have good antimicrobial activity against the bacteria *E. coli, Ps. Aeruginosa* and *MRSA.* This preliminary results indicates that these fungal metabolites contain antimicrobially active compounds that can be exploited for antibiotic production, and development. Further studies to elucidate the antibacterial active compounds can be initiated in that respect.

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AUTHORS CONTRIBUTIONS

Both EOB and MW-K contributed equally to this study. JG was responsible for conducting the antimicrobial studies. All authors read and approved the manuscript.

CONFLICT OF INTERESTS

None

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